

Phosphorus Determination in Waters and Extracts of Soils and By-Products: Inductively Coupled Plasma Spectrometry versus Colorimetric Procedures

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Introduction

The ability to measure phosphorus (P) concentrations in soils, water, and by-products is important from both an agricultural and an environmental perspective. One of the purposes underlying the development of soil testing was to identify soils that would likely have increases in crop yield with additions of P fertilizer. That use continues today and the procedures developed for identifying soils that would be responsive to additions of P fertilizer are also used to identify soils that have excessive amounts of P that might contribute to eutrophication of surface waters. The analysis of by-products, mostly animal manures, for P is important in our efforts to effectively manage P from concentrated animal feeding operations to minimize potential environmental impacts.

The methods used to measure P concentrations require that the P be contained in a liquid matrix. Therefore, for solid samples such as soils or by-products, the P must be brought into solution through a digestion procedure using acids or by extraction with a liquid such as water, weak salt solutions, or weak acids. Prior to analysis these solutions must be filtered to remove solid particles that would interfere with the analytical procedures. In the case of water, the sample may also be filtered through a specific type of filter and any P that passes through the filter is considered to have been dissolved in the original water sample. The water sample may also be digested prior to filtration and any P that is measured is considered an estimate of the total amount of P in the original sample.

The issue of filtration is important in P analysis. Filters are characterized by a pore size whereby particles in a liquid that are smaller than the pore size of the filter paper will pass through the filter paper. Even filter papers with a very small pore size will allow some solid particles to pass (sometimes called colloidal material) and P attached to those particles may or may not be measured in the analysis step. In general, the P contained in a filtered sample would exist as soluble inorganic or organic P species and P adsorbed to any colloidal material that has passed through the filter.

The possibility that P concentrations in extracts, digests, and water samples might be different when measured with inductively coupled plasma spectrometry (ICP) as compared to colorimetric procedures has been debated since the introduction of ICP instruments. The ability of ICP to measure several elements simultaneously has increased interest in multi-element extracts for routine soil testing and likely reduced the use of the colorimetric approach. However, colorimetric procedures offer some advantages, such as increased sensitivity and lower instrumentation costs, and it is unlikely that ICP will completely displace colorimetric procedures. The commonly held belief is that ICP would measure greater P concentrations than colorimetric procedures because the high temperature environment of the plasma would allow

the measurement of organic P compounds or other soluble P complexes that would not be measured colorimetrically. Differences in P concentrations measured by these two methods may have implications for agronomic and environmental P testing. The purpose of this paper is to discuss potential differences in P determination by ICP versus colorimetric procedures.

Magnitude and Characteristics of the Difference between ICP and Colorimetric P Analyses

The most common P colorimetric procedure in use, because of its high sensitivity, was developed by Murphy and Riley (1962). For purposes of this paper, colorimetric analysis refers to the Murphy and Riley method. The literature has sufficient examples of direct comparisons between ICP and colorimetric P determination to demonstrate that differences can clearly exist. Many of these papers discuss the Mehlich-3 (M3) method, which has been widely adopted in the eastern half of the U.S. The M3 extract is used as a multi-element soil extractant that matches well with the multi-element capabilities of ICP, although there are significant concerns about differences between ICP and colorimetric procedures with water samples as well as water or weak salt extracts of soils and manures. Most of the examples indicate that ICP will measure greater P concentrations than colorimetric methods, although examples can be found where ICP values are less than P concentrations obtained via a colorimetric procedure. For the sake of brevity, selected examples are presented here to illustrate the differences in various sample types.

Pittman et al. (2005) compared ICP and colorimetric P determination in M3 extracts of 6400 soil samples from Oklahoma. A significant correlation was found between the two methods (Fig. 1a) and the difference between the two methods was greater when considering only soils with $<60 \text{ mg kg}^{-1}$ M3 P (Fig. 1c). The intercepts of the lines shown in Figure 1 are close to zero and the slopes indicate that P concentrations determined colorimetrically will be 80 to 90% of those determined by ICP. The difference between the two methods is proportionally larger when the extractable P concentration in the soil sample is low ($<20 \text{ mg kg}^{-1}$, Fig. 2). Mallarino (2003) also found M3 results to be greater in Iowa soils when P was measured with ICP as compared to a colorimetric approach. Differences were large as indicated by a larger intercept and smaller slope (0.779), and the differences were also proportionally larger at low P values. Sikora et al. (2005) found the slopes of correlations between ICP and colorimetric P determination in M3 extracts of Kentucky soils were much closer to 1 (1.02 and 0.968 for two data sets) as compared to Pittman et al. (2005) and Mallarino (2003). However, when M3 extractable P was low and P fertilizer would be recommended, P concentrations determined by ICP were greater than colorimetric values more frequently than the reverse.

Jacoby (2005) evaluated 23 fields where manure had been applied either via a manure spreader or grazing cattle or both on feedyards and dairies. He compared Mehlich-3 P to colorimetric P and found a R^2 of 0.76 at $p < 0.01$ with a 47 mg kg^{-1} P intercept (Fig. 3). The colorimetric P was 55% of the P determined by ICP. The pH values ranged from 4.9 (only one below 5.5) to 8.6, with 14 of the 23 fields having a pH from 5.5 to 7.5. The range in colorimetric-P was from 31 to 472 mg kg^{-1} and ICP-P from 40 to 772 mg kg^{-1} .

Wolf et al. (2005) compared ICP and colorimetric determination of P in water extracts of manures and found that colorimetric P analyses were significantly greater, by an average of 7%,

than ICP analyses for swine, dairy, and broiler poultry manure samples (Fig. 4). Similar results were reported by Choate (2004) for 0.01 M CaCl₂ extractable P in biosolids (Fig. 5). These samples covered a wide range of P concentrations and colorimetric analyses were 13% greater than ICP. In contrast, recent results from a study conducted by SERA-17's soil and manure testing workgroup (Fig. 6), indicate that for manure and biosolids water extracts, ICP measurements are approximately 20% greater than colorimetric measurements. When those manures and biosolids were broadcast to soils and subjected to simulated rainfall to generate runoff, runoff water samples revealed a similar difference in ICP versus colorimetric measurements (approximately 20%).

Possible Causes for any Discrepancies between ICP and Colorimetric P Determinations

The possibility that ICP is able to measure organic forms of P that colorimetric procedures miss was investigated by Pierzynski et al. (2005). Standard organic P solutions containing glycerol 2-phosphate disodium salt hydrate, phytic acid, or 4-nitrophenyl phosphate bis (cyclohexylammonium) salt hydrate were analyzed by ICP and the Murphy and Riley procedure. Recovery from the solutions by ICP was essentially 100% for glycerol and phytic acid compounds and 88% for 4-nitrophenyl. Only 0 to 3.5 % was recovered by the colorimetric P procedure indicating that this approach was unable to detect P in the organic compounds (Table 1). The standard solutions were digested in potassium persulfate in an autoclave, and the recovery of P by ICP was near 100% for 4-nitrophenyl and phytic acid while decreasing to 86% for glycerol. Upon digestion and determination of P with the colorimetric procedure, recoveries increased to between 56 and 93%. These results suggested that laboratory digestion is not sufficiently rigorous to completely oxidize the organic P such that the P can be measured by the colorimetric procedure. Thus, if the difference between ICP and colorimetric P determinations is related to organic P species, then the digestion of extracts, digests, or water samples followed by colorimetric analysis may not be adequate to account for all of the P that can be measured with ICP, and merely digesting solutions prior to colorimetric analysis will not bring the two approaches into agreement. This is illustrated in Figure 7, which shows the correlation between P in M3 extracts as determined by ICP or the colorimetric approach in undigested or digested solutions. Digesting samples and determining P colorimetrically brings the slope of the correlation closer to one as compared to undigested samples, but does not completely compensate for the discrepancy. Incomplete digestion of organic P in the extracts cannot be ruled out based on the results shown in Table 1. Work coordinated by the NCR-13 soil-testing committee with Iowa, Minnesota, and Missouri soils (R. Eliason, G. Rehm, and A. Mallarino, unpublished data; Nathan et al., 2002) also showed that ICP determined near 100% of various organic P species added to M3 extracts while the colorimetric method recovered only trace amounts. These studies do indicate that ICP is fully capable of measuring P in some organic forms but do not allow us to determine with certainty if the presence of organic P species in extracts accounts for all of the difference between ICP and colorimetric P determinations.

Pierzynski et al (2005) also investigated the possibility of spectral enhancement of the P signal using ICP by measuring spectral emission intensity in a scan across the P wavelength in P standards and unknowns. Spectral enhancement would artificially increase P concentrations measured by ICP. No evidence of spectral enhancement was found.

Other possible explanations for the higher values obtained by ICP over colorimetric procedures in soil extracts include the presence of P-containing particulates (colloidal materials) not removed during the filtering process and/or the presence of soluble P complexes with iron (Fe), aluminum (Al) and/or calcium (Ca). The higher temperature environment of the ICP plasma could potentially result in the measurement of some fraction of these soluble or suspended P components that would not be measured by colorimetric procedures. However, we are not aware of any published studies that have specifically evaluated these effects.

Pittman et al. (2005) characterized 100 soil samples in greater detail that were then used to study relationships between other soil chemical characteristics and the difference between ICP and colorimetric P determination. Within this group, the slope of the correlation between ICP-P and colorimetric-P was 0.87 for all soils, 0.96 for soils with pH <5.5, 0.83 for soils with pH 5.5 – 7.5, and 0.88 for soils with pH > 7.5. Therefore, soil pH plays a role in the relationship between the two methods but does not explain the difference between them. This study also examined the potential influence of M3 extractable Al, Fe, and Ca; clay content; and soil organic carbon (C) content on the difference between colorimetric and ICP determination of P; but no significant relationships were found. Mallarino (2003) found a weak ($r^2 = 0.32$) negative relationship between M3-ICP minus M3-color and soil pH suggesting that the difference between the two methods increased as pH decreased. This is in contrast to the findings of Pittman et al. (2005). Similarly, the difference between the two methods decreased when soil organic C increased ($r^2 = 0.16$), which suggests that organic P may not account for all of the difference between ICP and colorimetric approaches if one assumes that extractable organic P would increase with increased soil organic C. Studies in Minnesota showed the difference between the methods tended to be smaller with increased pH but did not change significantly with increased organic C (Elliason et al., 2001). Studies in Missouri found no consistent relationships between the additional P measured with ICP and manure applications or soil organic C (Nathan and Sun, 1998; Nathan et al., 2002).

The colorimetric procedure determines the P concentration by measuring the amount of light at a specific wavelength that is absorbed by a colored complex formed with P. Greater absorption corresponds to a greater P concentration. Reports of colorimetric analyses being greater than ICP are thus far confined to water extracts of organic by-products and it is not known if this situation exists with other sample types or extracts. Wolf et al. (2005) hypothesized that substances were extracted with the manure also absorbed light at the specific wavelength and that did not contain P. Such substance would induce a positive bias in the colorimetric analyses as compared to ICP. The findings of SERA-17's soil and manure testing workgroup (Fig. 6), where colorimetric and ICP determinations were run by the same laboratories as in Wolf et al. (2005), indicate that the role of color interference in inflating colorimetric P measurements is not universal.

The possibility of human error cannot be discounted and there are a variety of ways in which human error can induce a positive or negative bias in results. Preparation of standards, calculation errors, and dilutions are three common factors that can influence results.

It is likely that organic P species play a role in the discrepancy between ICP and colorimetric P analyses when ICP results are greater and that other as yet unidentified factors may also play a role. We currently have little insight into what may cause colorimetric P analyses to be greater than ICP results for water extracts of organic by-product materials. Overall, this is an area of needed research from an academic point of view. On the practical side, the cause of the discrepancy is less important than the magnitude of the discrepancy.

Practical Significance

Phosphorus analyses of soils, waters, and manures can be used for a variety of purposes including making P fertilizer recommendations; estimating the risk of P loss in runoff; or assessing actual P losses from the landscape. The interpretation of the results may be based upon P analyses determined colorimetrically or by ICP. A common example would be soil test calibrations which have historically been based on colorimetric P analyses. Clearly, if a method other than the one used as a basis for the interpretation is employed, then significant errors may occur. A reasonable worst case scenario would be a decrease in recommended P fertilizer proportional to the enhancement of the soil test P results measured by ICP as compared to recommendations based on colorimetric analyses. To avoid misinterpretation of soil fertility results obtained by ICP, it has been suggested that equations, such as those shown in Figure 1, be used to convert ICP data back to colorimetric analyses. However, obtaining the appropriate correction factors or equations is no trivial matter as these relationships are potentially influenced by soil type, and factors such as soil pH or organic matter content. Until the specific factors contributing to the higher values obtained by ICP over colorimetric procedures have been more clearly defined, cautions should be excised when using a regression approach for converting ICP to colorimetric-equivalent values since the relationships could be different for different regions. An alternative approach is to develop separate calibration sets for colorimetric versus ICP determinations as recommended by Mallarino (2003). Noting that the differences between ICP and colorimetric P determinations were not constant across Iowa soils and that they were only weakly correlated to soil organic matter and pH, Mallarino demonstrated that a different field response-based calibration for M3 colorimetric versus M3 by ICP was merited (Fig. 8). Additional unpublished research for other crops resulted in the publication of Iowa soil-test interpretations for colorimetric and ICP versions of the M3 extractant (Sawyer et al., 2002). Iowa considers the M3 extractant with ICP or colorimetric determination of extracted P as two, clearly different soil tests.

The issue is less clear when using P analyses as part of a determination of the risk of P loss from the landscape. The common tool for this purpose would be one of the P Indices used in the U.S., where P analyses may be a factor in varied ways including soil test P data or water extractable P for manures (Sharpley et al., 2003). If the method of P analysis is not specified for a given P Index, then it is not possible to correct for any possible discrepancies as may be possible for fertilizer recommendations. The typical output from a P Index is a relative ranking of risk for P loss from the landscape in categories of very low, low, medium, high, and very high. It is possible that the use of one method for P determination over another may result in a shift up or down one category and that change may have important ramifications. For example, a lower category may allow a producer to apply animal manures on a N basis instead of a P basis. If the

method of P analysis is specified for a given P Index, then it may be possible to correct any P analyses to the specified method and avoid any possible variations in the P risk loss category that is determined for a given site. The freedom to use any method of P analyses or the specification of a particular method are means to build flexibility and varying degrees of conservatism into the process. Not specifying a method may allow a shrewd producer to select the method that is to his or her greatest advantage. Specifying a method may force the most or least conservative interpretation of the P Index. A recent modification of the Iowa P Index specifies that both the M3-colorimetric and M3-ICP soil tests can be used as source factors for the index but different equations are used for these tests when estimating P in surface runoff or subsurface drainage (Mallarino et al., 2005).

If one assumes that ICP would indicate greater P concentrations in samples of surface runoff as compared to the colorimetric approach, then using ICP would present the worst case scenario with regard to runoff losses of P. It is difficult to interpret the potential ramifications of the method of P analysis beyond this given our lack of ability to interpret edge of field P losses on a larger watershed scale. Samples of water collected from streams or lakes may be used to estimate compliance with total maximum daily load (TMDL) requirements or the degree of impairment from P, and would also present a worst case scenario if ICP were the method employed.

Some states or regions have heavily relied on soil test P threshold values to decide whether animal waste is allowed to be applied or not. It is particularly important to specify a method of sampling and analysis to minimize unnecessary confusion.

Summary and Conclusions

The method of P determination used in water samples and extracts of soils and by-products can influence the reported concentrations. For most sample types, ICP is slightly greater than colorimetric analyses, but the two methods are well correlated for high P samples. However, extreme caution needs to be used for samples with low P concentrations (e.g., 60 mg/kg extractable P), as the difference may be as great as 5 fold. Water extracts of manures analyzed using colorimetric procedures may be greater than ICP by as much as 15%. The reasons for the differences have not been fully investigated. When ICP results are greater than colorimetric values, it appears that the ability of the ICP to measure organic P species plays a role.

The difference between the two methods can be important when interpreting results. Most fertilizer recommendations are based on colorimetric P analyses and the use of ICP may produce different recommendations. The use of correction factors needs further investigation. The impact is less obvious when using P analyses as part of a P Site Index. The use of one method may cause the estimated risk of P loss to be slightly greater or less than that predicted using another approach. For water samples from surface runoff, lakes, or streams, the use of ICP would likely estimate the worse case scenario in terms of the potential loss or impact of P.

SERA-17 Committee Recommendations:

- 1) The method of P analysis should always be clearly stated when presenting analytical results.
- 2) If P data collected with a particular method supports subsequent interpretation of P analyses, then that method should be clearly identified.
- 3) More research is needed to determine the sample types and range of P concentrations for which significant differences in results might be obtained with ICP and colorimetric methods.
- 4) More research is needed to identify the reasons for discrepancies between ICP and colorimetric P analyses.
- 5) The use of regression analysis to convert ICP to colorimetric-equivalent values needs further evaluation for a variety of conditions. Until additional research is completed, extreme caution is warranted whenever such conversion procedures are used.

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Table 1. Recovery of P from undigested or digested solutions of organic P compounds using ICP or the Murphy and Riley method. Solutions contained either 15 or 20 mg P/L. Source: Pierzynski et al. (2005).

Compound [†]	----- Digestion/Analysis Method -----			
	Undigested/ICP	Undigested/Color	Digested/Color	Digested/ICP
	----- % recovery -----			
4-nitrophenyl	88	0	78	100
Glycerol	101	0	56	86
Phytic acid	96	3.5	93	101

[†]4-nitrophenyl = 4 nitrophenyl phosphate, bis (cyclohexylammonium) salt hydrate; glycerol = glycerol 2-phosphate disodium salt hydrate

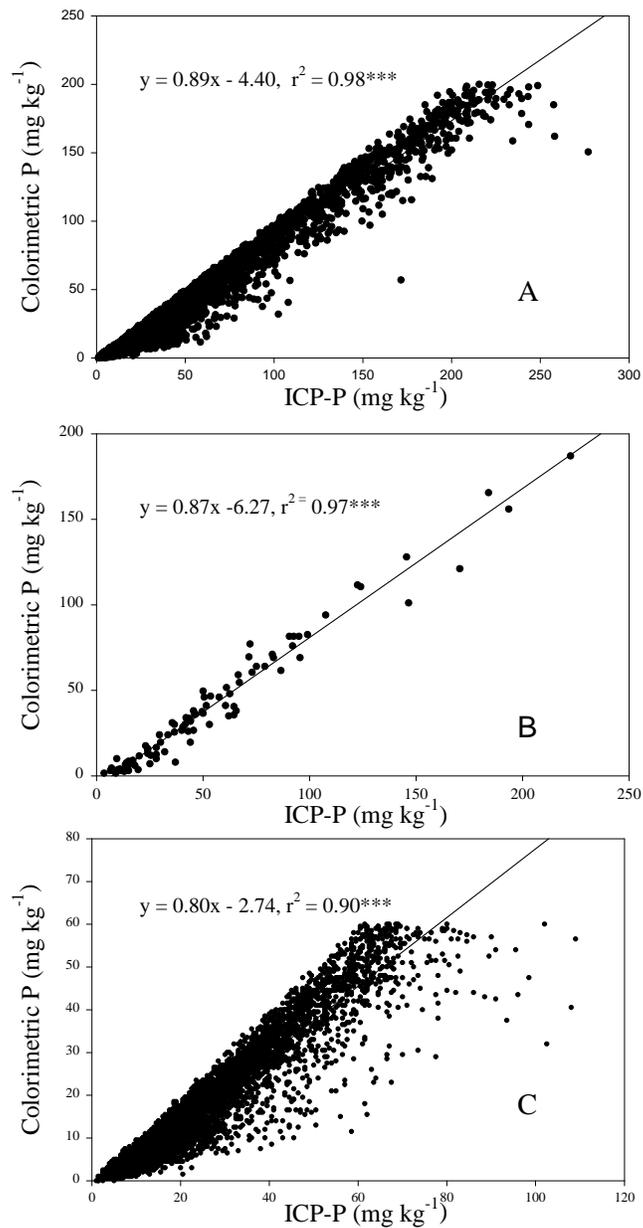


Figure 1. The relationship between Colorimetric P and ICP-P for (A) 6400 soil samples received by Oklahoma State University Soil Testing Laboratory (B) 100 characterization soils and (C) 5400 soils with colorimetric P < 60 mg kg⁻¹. ***p < 0.001.

From Pittman et al. (2005).

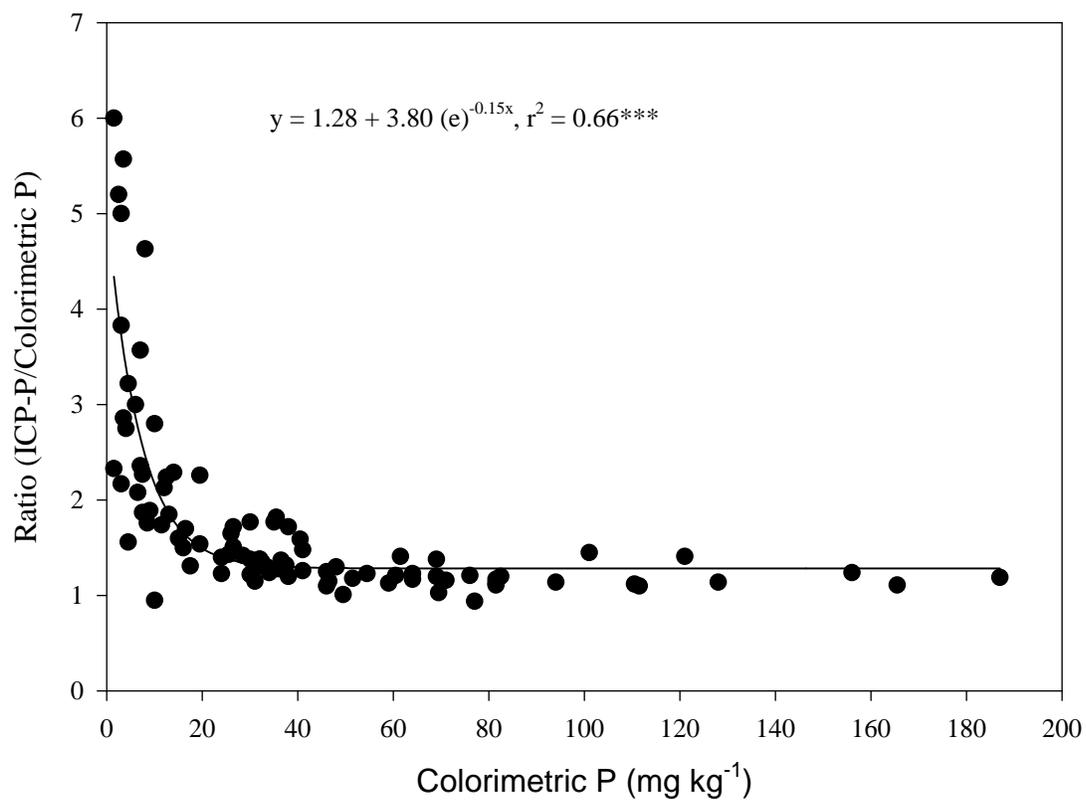


Figure 2. The relationship between the ratio of ICP-P and Colorimetric P and Colorimetric P for the 100 characterization soils. *** $p < 0.001$.

From Pittman et al. (2005).

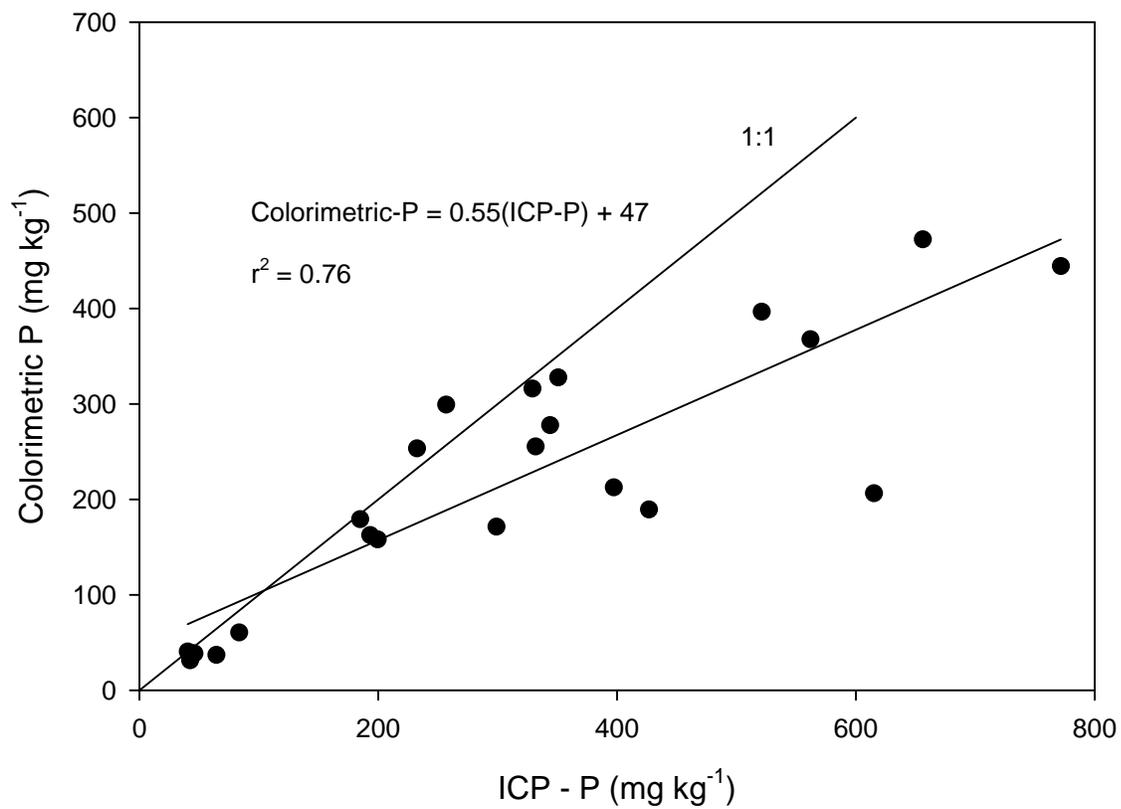


Figure 3. ICP versus colorimetric P analysis for Mehlich-3 extracts of manure-amended soils in Texas. Source: Jacoby (2005).

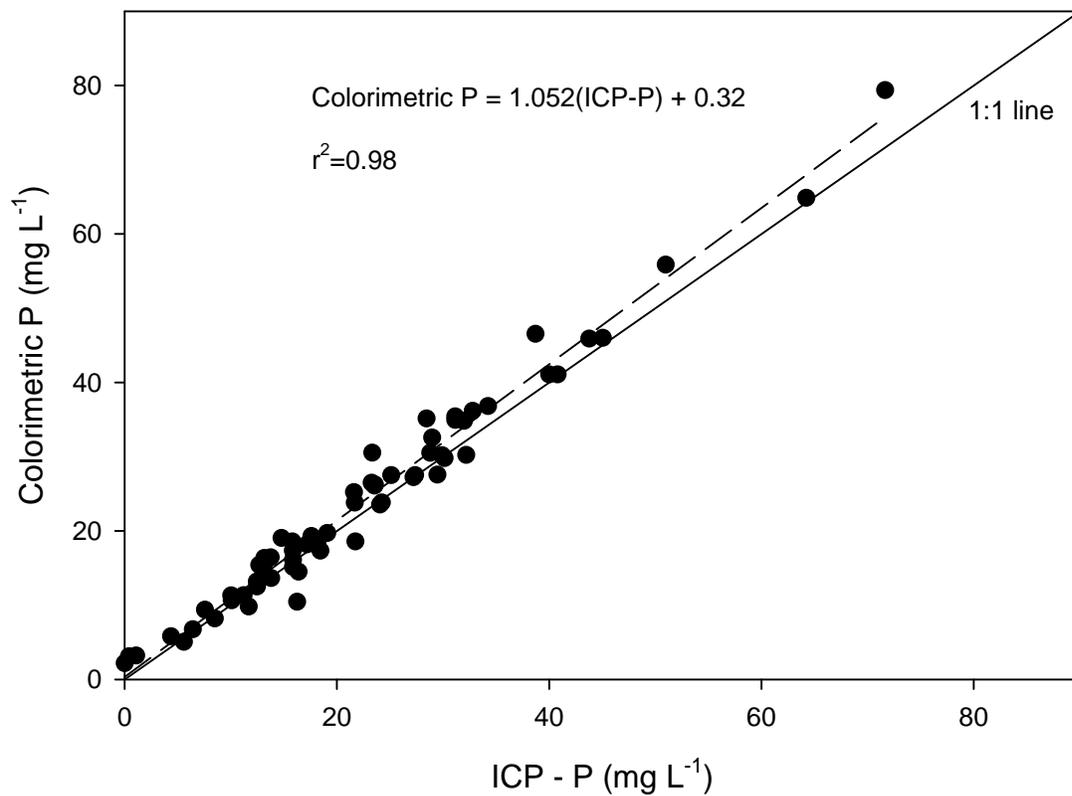


Figure 4. ICP versus colorimetric determination of P in water extracts of manures. The solid/solution ratio was 1:200. Redrawn from Wolf et al. (2005).

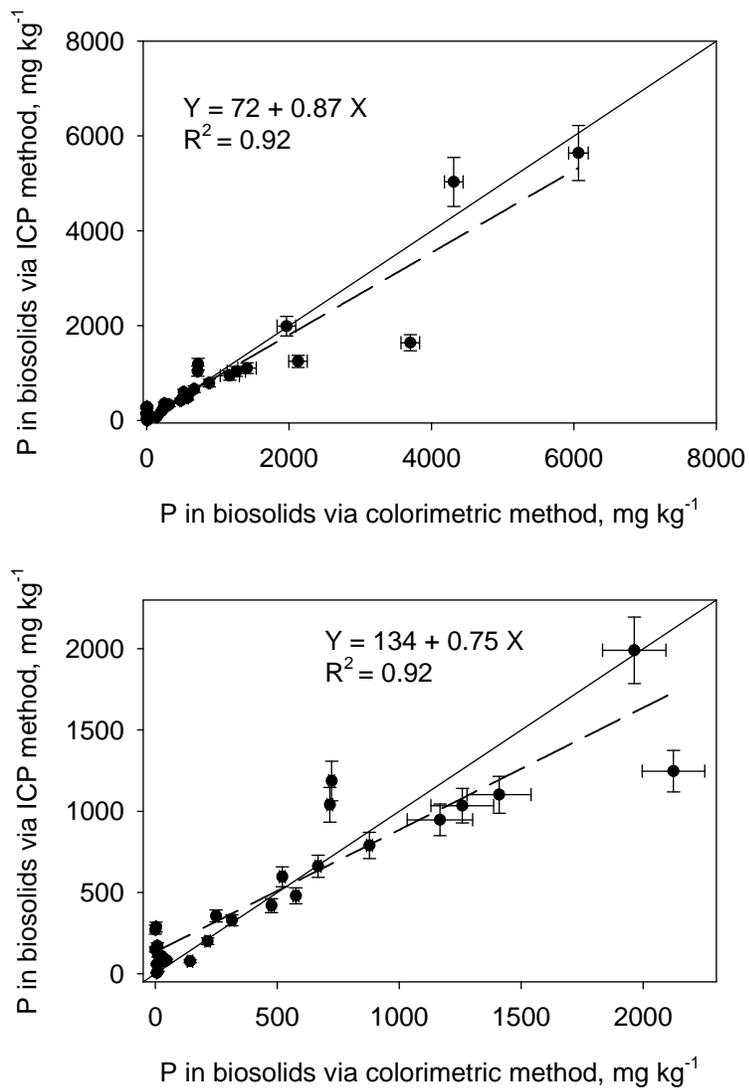


Figure 5. Phosphorus extracted with 0.01M CaCl₂ from 30 samples of oven-dried municipal biosolids. Source: Choate (2004). Only difference in graphs is scale (0 to 8000 above and 0 to 2500 below)

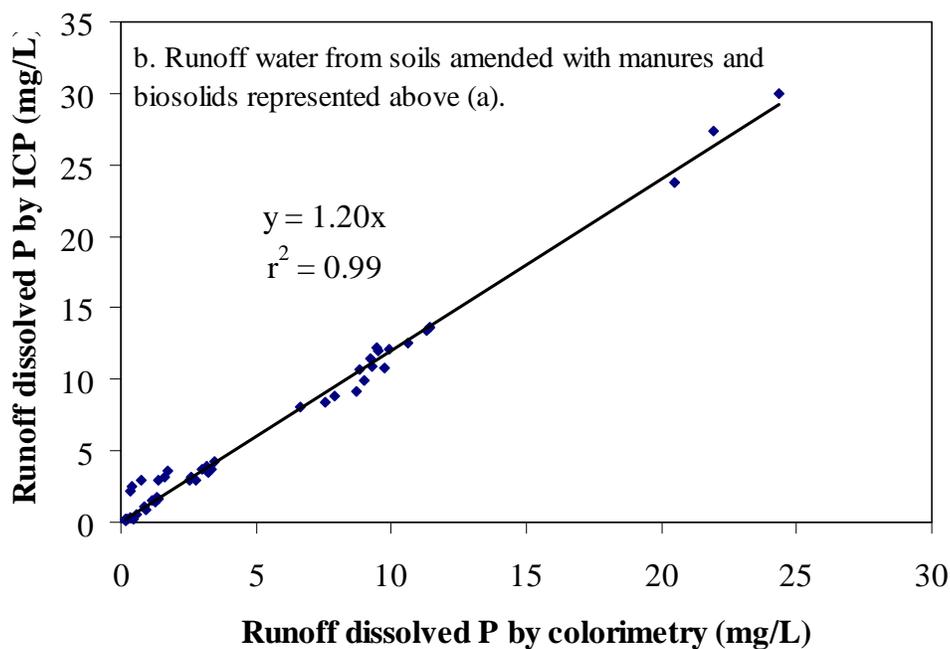
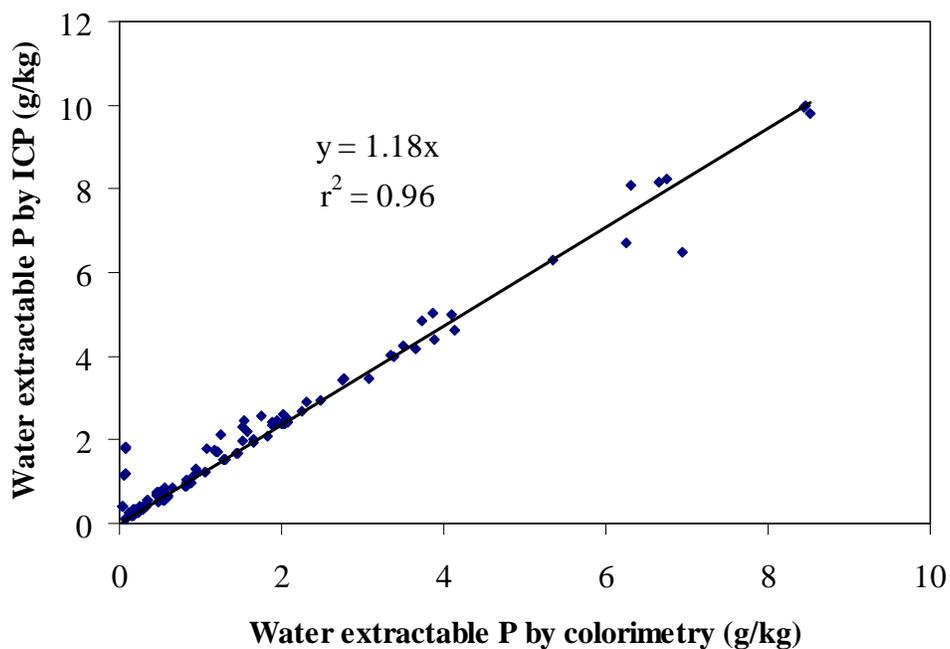


Figure 6. Relationship of P determined by colorimetry and ICP in manure and biosolid water extracts (a) and runoff water from soils broadcast with manures and biosolids (b). Note that regressions have been forced through the origin to counter effect of outliers on slope. Source: SERA-17 soil and manure testing workgroup, unpublished data.

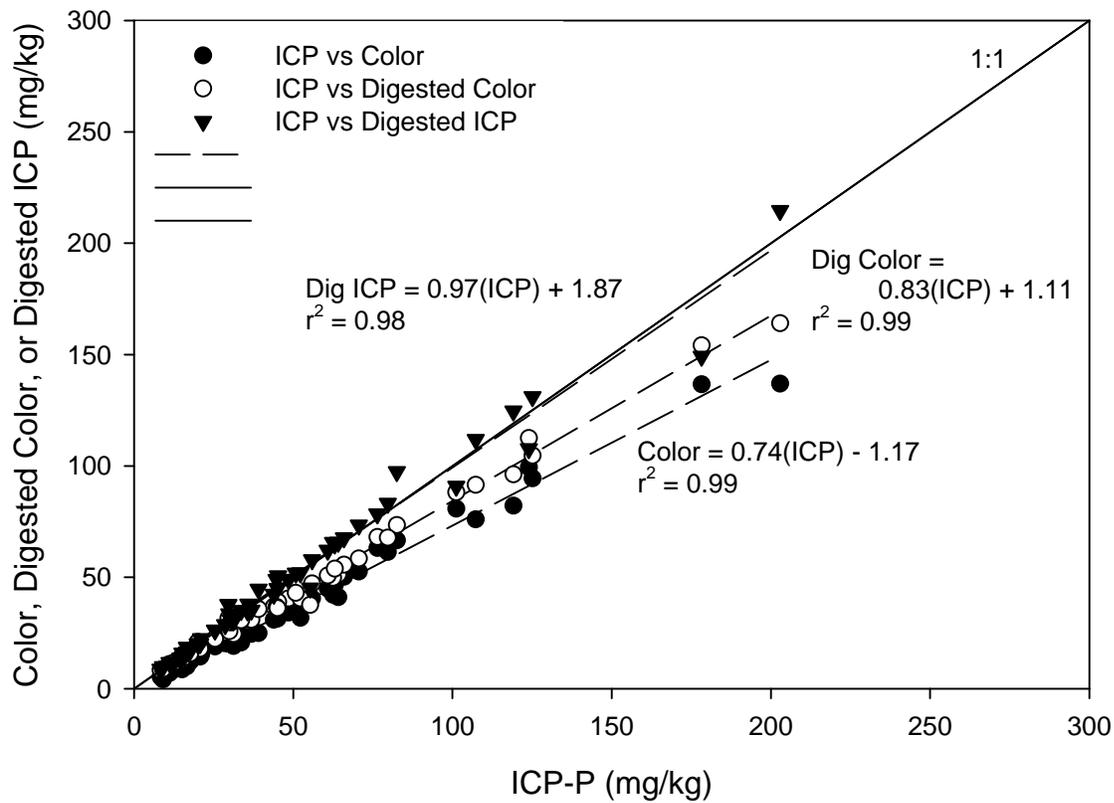


Figure 7. Phosphorus in the Mehlich-3 extracts of 50 Kansas soils, with or without persulfate digestion, as measured by ICP or colorimetric procedures. Source: Pierzynski et al. (2005).

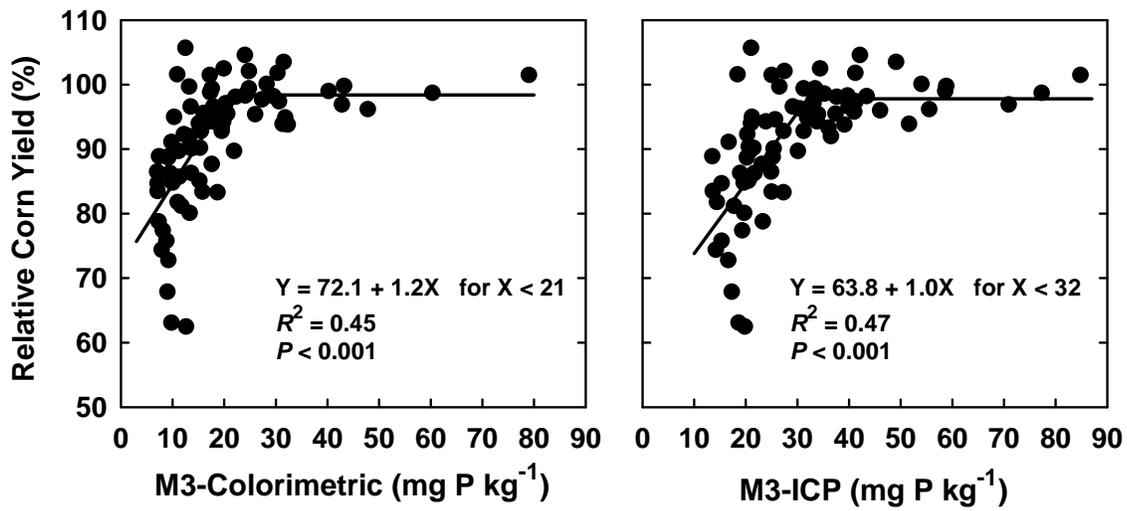


Figure. 8. Relationships between relative yield response of corn and soil-test P in Iowa soils measured using the Mehlich-3 extractant and determining extracted P with colorimetric and ICP methods (adapted from Mallarino, 2003).